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## PROGRESS OF ACTIVITIES UNDER THE COLLABORATIVE PROJECT: SUMMARY

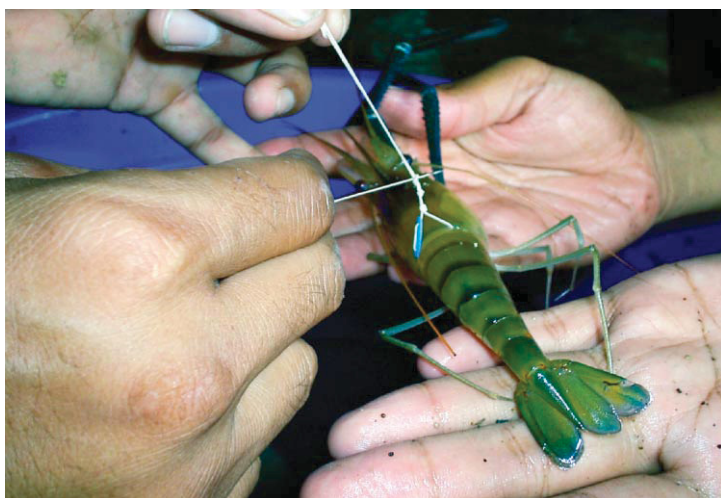
### Evaluation of the Growth Rate of GI Macro II Strain in Different Locations (Indonesia)

Assisted by Mr. Maskur as his co-worker, Dr. Estu Nugroho reported on the final results of their study which was conducted in Indonesia. In the study, selective breeding program has been conducted to improve the freshwater prawn from synthetic population gathered from numerous breeders collected from the waters of Tanjung Air (Bekasi) collected in February 1995, Kalipucang (Ciamis) collected in June 1996, and Musi (Palembang) collected in May 1997. Index selection was applied to these subpopulations to improve the edible portion trait of the freshwater prawn.

The synthetic population was constructed from the two subpopulations that were then added to the subpopulation from Musi. Family selection was applied to the synthetic population. In 2001, GI Macro or the Genetically Improved *Macrobrachium rosenbergii* has been developed and released to the farmers. Based on the farmers' feedback, the sustainability of GI Macro culture was evaluated in different locations and verification studies were conducted to improve the growth rate. From the 1800 selected breeders taken from 18 families of GI-macro II, 25% were used as a selected line while the 25% residual to average size was used as the control line (50 male and 50 female per each family).



Mr. Maskur (left) and Dr. Estu Nugroho (right) presenting the results of their study in Indonesia

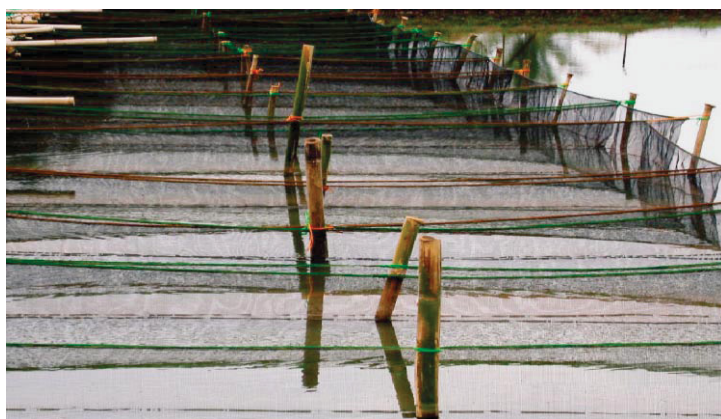


Plastic tagging of freshwater prawn breeders

Each family, differentiated by plastic tags, comprised about 50 breeders selected for the mass spawning. A total of 900 breeders from each line (selected and control line) with 1:1 male and female ratio were mass spawned separately in 200 m<sup>2</sup> concrete pond for one month. Mature eggs (chocolate color) from seventy female breeders (selected line) and from 60 female breeders with same condition from the control line were collected for hatching.

About 24 thousand larvae from the selected and control lines have been collected and reared in different concrete tank provided with bamboo shelters. The resulting PL10 larvae were reared up to 3-5 cm in a concrete tank at 400 PL/m<sup>2</sup> and fed four times daily with natural feed (i.e., *Moina* and *Daphnia*), and pellet feed containing 38% protein at 20% of body weight. *M. rosenbergii* juveniles at 0.3 g ave weight were reared in hapa in the pond at 85 juveniles/m<sup>2</sup> in three different locations (Sukamandi, Cibalagung and Cijeruk).

The results indicated that the average total length and survival rate of selected line after over one month culture was higher than the control. The average total length and survival rate of GI Macro varied between batches 1 and 2, indicating that the population of GI Macro has wide variance. The average total length and weight of GI Macro from the selected line was better than the control in three different locations. Even the selected line of GI Macro in Sukamandi grew slower than the control in the first six weeks. In Cibalagung and Cijeruk, the selected line of GI Macro grew faster than the control after 12 weeks of rearing. The best harvest weight was the GI Macro reared in Cibalagung and followed by Sukamandi and Cijeruk. This showed that rearing GI Macro is more suitable in low to moderate level than in high sea level area.



*Rearing of freshwater prawn juveniles in hapa nets in one of the three different locations*

It should be noted that the water sources of the three locations are: natural water in Cijeruk, channel water from paddy field in Cibalagung as well as in Sukamandi, which might have also influenced the growth rate of the GI Macro.

The length gain of the GI Macro reared in three different locations for three months was positive. However, GI Macro reared in Cijeruk only showed positive tendency during the research period, while the negative value of length gain was still observed in GI Macro reared in Sukamandi and Cibalagung. A similar result was also obtained in the weight gain of the GI Macro. The positive value of weight gain was observed in GI Macro reared in Cijeruk, while a negative value was observed in Sukamandi and Cibalagung. The selection activity has more effect on weight gain than in the length gain. The highest weight and length gain of the GI Macro in each location was 52% and 14% (Sukamandi) after 8 weeks, 73% and 15% (Cijeruk) after 8 and 6 weeks, and 35% and 8% (Cibalagung) after 6 and 8 weeks. The wide variance of GI macro weight and length was also observed genetically using DNA Markers. Thus, another selection activity should be conducted to improve their variability as a pure line. The heritability and response selection will also be done in the next phase of the study.

### **Selective breeding program for genetic improvement of *M. rosenbergii* (Thailand)**

*Growth comparison of three *M. rosenbergii* stocks and their reciprocal crosses grown in four environments*



*Dr. Supattra Uraiwan presenting the results of one study conducted in Thailand*

In her report on the growth comparison of three *M. rosenbergii* stocks, Dr. Supattra Uraiwan of the Aquatic Animal Genetics Research and Development Institute (AAGRDI) of Thailand explained that three stocks (AAGRDI, FARM (Petchaburi Farm) and WILD) of *Macrobrachium rosenbergii* were used for AAGRDI's selective breeding program. The crosses of these three stocks were evaluated in terms of performances and genetic variations before selective breeding program took place. AAGRDI's selective breeding program has two main parts: (1) evaluation of growth performance of the three stocks and their reciprocal crosses grown under four environments, and (2) improvement of the economic traits of the best cross adopting a suitable selection procedure.

In the preparation of the parent generation, fifty pairs of *M. rosenbergii* from each three stocks were collected for the base population. The stocks were spawned and their respective larvae were reared separately in three 20 m<sup>2</sup> concrete ponds. The growth performances of *M. rosenbergii* from three stocks were observed from August 2003 to January 2004.

The results illustrated that the *M. rosenbergii* of the "AAGRDI" stock performed better than the "WILD" and "FARM" stocks at an average of 4% and 9-15% in lengths and weights, respectively. In addition, allozyme electrophoresis has been carried out to estimate genetic variabilities (heterozygosity and number of alleles per locus) of the three stocks.

The results further illustrated that the genetic variabilities of *M. rosenbergii* from the present study are similar to those of *M. rosenbergii* from the natural waters {No of alleles 1.30 (1.29-1.33), heterozygosity 0.032(0.027-0.036)}. It was also observed that there was no difference on genetic diversity between these three stocks.





The AAGRDI stock (left), FARM stock (center) and WILD stock (right) used in the study

The reciprocal cross of the three stocks was conducted from November 2004 to August 2005 to establish nine cross-lines. The crosses indicated by male and female parents of each cross, starting with the male parents followed by the female parents, are as follows: AAGRDI x AAGRDI, WILD x WILD, FARM x FARM, AAGRDI x FARM, FARM x AAGRDI, AAGRDI x WILD, WILD x AAGRDI, FARM x WILD and WILD x FARM. Each cross was produced from 10 pairs of male and female *M. rosenbergii*. The hatching and nursing period took place at the AAGRDI and the Petchaburi Fisheries Test and Research Center in different periods while the post larvae were transferred for the performance growth test in four environments (AAGRDI, Chumphon Fisheries Test and Research Center, Buriram Fisheries Test and Research Center, and Uttaradit Fisheries Test and Research Center).

The nine crosses were reared under the above environments for 8 months. Similar experiment procedures such as stocking density, feeding regime and measurement schedules were used in all treatments. The stocking rate of 10 and 1 prawn/m<sup>2</sup> was used from the 1<sup>st</sup> to the 4<sup>th</sup> month and the 5<sup>th</sup> to the 8<sup>th</sup> month, respectively. The prawns were fed with commercial pellet shrimp feed three times a day at 3.4% of body weight. Because of the difference in starting time, the growth in lengths and weights of *M. rosenbergii* were monitored after the 2<sup>nd</sup> month for the AAGRDI and the Chumphon Fisheries Test and Research Center, and after the 3<sup>rd</sup> and 4<sup>th</sup> month for the Buriram and Uttaradit Fisheries Test and Research Centers, respectively.

In the AAGRDI environment, the cross WILD x WILD gained the highest in lengths and weights. It was significantly higher in lengths and weights than those of the AAGRDI x AAGRDI and the FARM x FARM, respectively. At the Chumphon Fisheries Test and Research Center environment, the cross WILD x AAGRDI had the highest in lengths and weights. It was significantly higher in lengths and weights than those of the AAGRDI x AAGRDI. At the Buriram Fisheries Test and Research Center environment, the cross AAGRDI x FARM gained the highest in lengths, significantly higher than those of the AAGRDI x AAGRDI, the FARM x FARM and the WILD x WILD.



At the Uttaradit Fisheries Test and Research Center environment, the cross AAGRDI x FARM had the highest in lengths and weights, significantly higher than those of the AAGRDI x AAGRDI, the FARM x FARM and the WILD x WILD.

AAGRDI facilities (left), and below are the facilities at Buriram Fisheries Test and Research Center (left), Chumphon Fisheries Test and Research Center (center) and Uttaradit Fisheries Test and Research Center (right) which were used in the study





*Allozyme marker based comparison on genetic variation among M. rosenbergii populations produced from a cross-breeding system of three different stocks in Thailand*

According to Thailand's Dr. Panom Sodsuk, a selective breeding program on the giant freshwater prawn including studies on improving the growth performance on the domesticated strain was also carried out at the Aquatic Animal Genetics Research and Development Institute (AAGRDI) of the Department of Fisheries of Thailand.

Dr. Sodsuk added that AAGRDI has already developed a domesticated and genetically improved stock of *Macrobrachium rosenbergii* for two generations. A wild stock has also been domesticated under the hatchery condition of the AAGRDI for one generation.

Meanwhile, domesticated stocks from private hatcheries were also developed. But there is still a need to develop another improved stock of the species basically from the two stocks of AAGRDI, the genetically improved and the wild, together with the domesticated stock from a good private hatchery so that the new created stock, which will be used as base population for further selective breeding program, would have been developed with higher genetic diversity.

Specifically, the study aims to: (1) evaluate genetic variation (in terms of genetic variabilities as per locus averages of observable heterozygosities and number of alleles) of nine crosses from the above three mentioned stocks (the genetically improved by AAGRDI, the wild, and the private farm) of *Macrobrachium rosenbergii*; (2) apply polymorphism system of allozyme markers in the evaluation; (3) compare the evaluated genetic variation among the nine crosses to see differences; and (4) Use the informations of genetic variation evaluated, together with the performances, to choose the best cross for further selective breeding program in appropriate area. About 40-60 individuals of both sexes of each of the three stocks (AAGRDI, WILD and FARM) and each progeny population of nine crosses were sampled. Pleopods from each individual were cut and collected in separate microtubes. All pleopod samples in microtubes were preserved for further molecular analysis of allozyme markers. All preserved samples were electrophoretically analysed at 19-25 allozyme loci following a procedure already established by AAGRDI.

All allozyme data from the laboratory analyses were collected and calculated as per locus averages of heterozygosities (H) and number of alleles (NoA) for genetic variation evaluation. The work was done using a particular software for population genetics studies (BIOSYS release 1.7.) The genetic variations, as per locus averages of heterozygosities and number of alleles of the nine crosses were compared using the software SYSTAT. Results indicated that the amounts of genetic variation, evaluated as per locus averages of heterozygosities and number of alleles, of the three initial stocks and all nine crosses showed no significant differences among the three initial stocks, as well as the nine crosses, both by heterozygosities and number of alleles. The appearance of heterozygosities and number of alleles both in the three initial stocks and in the nine crosses were close to those of the natural stocks studied before at the AAGRDI. Based on the resulting heterozygosities and number of alleles from the study, certain best crosses could be chosen for appropriate culture area.



*Dr. Panom Sodsuk reporting on the progress of the allozyme marker study on the freshwater prawn conducted in Thailand*



*Representatives from Chumphon Fisheries Test and Research Center, Buriram Fisheries Test and Research Center, and Uttaradit Fisheries Test and Research Center who helped implement the project in Thailand*





## Morphometric Characterization and Performance Evaluation of Different *Macrobrachium* stocks and closely related species in the Philippines (Philippines)

The Philippine Bureau of Fisheries and Aquatic Resources (BFAR), the Mindanao State University (MSU) and AQD's Binangonan Freshwater Station jointly conducted preliminary studies on the genetic characterization, domestication and improvement of *Macrobrachium rosenbergii* stocks in the Philippines in order to improve the aquaculture production of the freshwater prawn. A study on collection, identification and validation of *Macrobrachium* samples was conducted by BFAR and AQD.

In addition, two studies on the evaluation of growth performance of two strains of *M. rosenbergii* in lake environment: reproductive efficiency of two *M. rosenbergii* stocks at different protein levels, and preliminary observations on the juvenile performance of *Macrobrachium rosenbergii rosenbergii* and *M. rosenbergii dacqueti* in hapa net cages at different stocking densities were conducted by AQD. A study on *Macrobrachium rosenbergii* and other indigenous species in Mindanao and Visayan islands (Philippines) was conducted by MSU.

### Collection, Identification and Validation of *Macrobrachium* Samples

Twelve species of *Macrobrachium rosenbergii* and other species that closely resemble the giant freshwater prawn have been caught from 25 commercial fishing grounds in the Philippines. The identities of local species, wild or hatchery-bred were validated. The western subspecies of Malaysian, Indonesian and Thai stocks of *Macrobrachium rosenbergii* and eastern subspecies found mainly in the Philippines were differentiated. The study was conducted in order to: (a) taxonomically validate the identity of the existing hatchery-bred and wild *Macrobrachium rosenbergii* stocks used in commercial aquaculture and *Macrobrachium* research in the Philippines; (b) identify possible sources of good quality *Macrobrachium rosenbergii rosenbergii* in the Philippines (particularly in Visayas and/or Mindanao) which can be used for domestication and selective breeding programs; and (c) characterize other indigenous *Macrobrachium* species that may have some aquaculture potential.



The team from the Philippines who are involved in the project

The distinguishing characteristics of each species which were identified from the samples collected are briefly described below:

*Macrobrachium rosenbergii* (giant river prawn)

**Eastern form:** *M. rosenbergii rosenbergii* (De Man, 1879)

**Western form:** *M. rosenbergii dacqueti* (Sunier, 1925)

AQD's Dr. Ma. Rowena Eguia reported that there are several subtle differences between these two forms or subspecies of *Macrobrachium rosenbergii*. However, the main difference between them is the basal crest of the rostrum. The basal crest of the *M. rosenbergii dacqueti* is higher than that of the endemic *M. rosenbergii rosenbergii*. The body of the *M. rosenbergii dacqueti* is dark green to grayish blue with longitudinal streaks of darker and lighter color while that of the *M. rosenbergii rosenbergii* has some pattern.



AQD's Dr. Rowena Eguia reporting on the morphometric characteristics of various *Macrobrachium* strains in the Philippines



Dr. Eguia added that the second legs of *M. rosenbergii* are very large, robust and of same size. In adult males, the entire second leg is densely covered with spines and sharp tubercles. The giant river prawn is the largest known *Macrobrachium* species and from the samples analyzed, the largest adult individuals were obtained from Dinas and Tambulig (in Mindanao in the Philippines), with total lengths of 23.6 cm. and 23.5 cm, respectively.

*The basal crest of the wild-sourced M. rosenbergii rosenbergii (bottom) is higher than that of the hatchery-bred M. rosenbergii dacqueti (top).*

#### *Macrobrachium equidens*

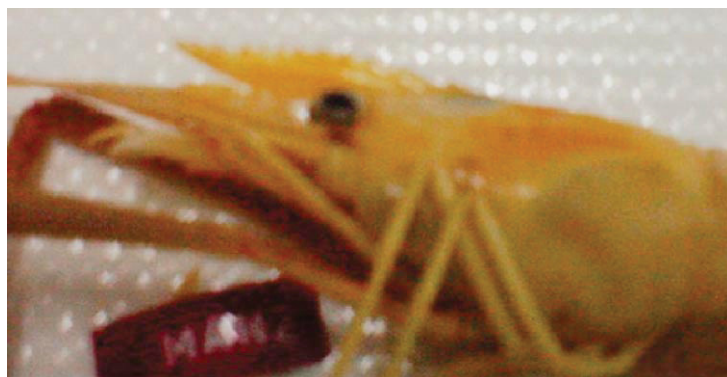
(Rough river prawn or estuarine prawn)

This prawn (right) is rarely found in pure freshwater, as they normally thrive in lower parts of streams, river mouths, estuaries where the water has a higher salinity (brackishwater) and observed to breed in brackish and sea water. From the samples analyzed, 13 individuals from Tambulig were *M. equidens* (2.2 cm average carapace length, 9 cm. total length, 7.2 cm. body length, and 9 upper and 5 lower rostral teeth. The rostrum of the *M. equidens* almost always exceeds the distal end of the antennal scale. The large second legs are marbled like tortoise shell.



*Macrobrachium equidens* sample from Tambulig in Mindanao

#### *Macrobrachium mamillodactylus* (knobtooth prawn)



*M. mamillodactylus* from Tambulig (in Mindanao, the Philippines)

The distinct feature of this species (left) is the shape of the rostrum: the tip of the rostrum does not reach the distal end of antennal scale. The large second legs are longitudinally striped and the fingers of the second legs are not covered by soft short hair but have rows of tubercles along the cutting edges. Samples of *M. mamillodactylus* were obtained from Tambulig and Mangagoy in Mindanao. The average measurements of the samples were: 3.6 cm. carapace length, 12.1 cm. total length and 10 cm body length.

#### *Macrobrachium latidactylus* (scissor river prawn)

This species is found mainly in estuarine and inshore marine waters. Adults are commonly found in tidal freshwater but larval development occurs in the sea or brackishwater environment. This species has unequal size of the large second leg even in young specimens. *M. latidactylus* samples were obtained from Mangagoy and Lake Lanao (in Mindanao). The largest sample was obtained from Mangagoy (2.8 cm carapace length, 7.1 cm body length and 8.1 cm total length).



*M. latidactylus* samples from Mangagoy (left) and Lake Lanao (right)





### *Macrobrachium lanceifrons* (Philippine river prawn)

This species is locally known as “hipon tagunton”. It is one of the commercially important prawn species in Laguna de Bay as it is used for human consumption and for duck food.

The tip of the rostrum of *M. lanceifrons* is slightly curved upwards in full grown individuals but straight in the young. The second pair of walking legs or chelipeds is equal in length in young specimens but unequal in fully grown.

Fully grown males are best distinguished from fully grown females by the length and shape of the second leg or cheliped. In the male, this is longer and is provided with felted hairs on the mobile finger.



Samples of *M. lanceifrons* from Lake Mainit



BFAR's Dr. Melchor Tayamen reporting on the collection of freshwater prawn species in Luzon area

Samples of *M. lanceifrons* were identified from the collection obtained in Lake Mainit (in Southern Mindanao, Philippines). The Lake Mainit samples had a 1.5 cm average carapace length, 4.3 cm. body length and 5.3 cm total length. More samples should be collected and observations on the breeding behavior and distribution in specific habitats/microhabitats should be done in order to determine the exact nature (that is whether they are found naturally in the collection areas as wild stocks or as accidental or intentional introductions from hatchery populations). Samples especially of *M. rosenbergii dacqueti* and *M. rosenbergii rosenbergii* will be processed for genetic marker analysis in 2006. Samples of these two subspecies will be collected from various populations to determine the level of intraspecific variation among the populations and their phylogenetic relationships.

### *Evaluation of Growth Performance of Two Strains of M. rosenbergii in Cages in Laguna de Bay*

*Macrobrachium rosenbergii* from two separate stocks (CAL- progenies of the native strain from Calumpit, Bulacan; and BFAR- progenies of the strain from BFAR, originally from Thailand) were reared in net cages in Laguna de Bay at a stocking density of 15 prawns/m<sup>2</sup>.

Two runs have been conducted for five months, the first run was from October 2004 to March 2005 and the second from April to September 2005.

In the first run, CAL showed significantly better specific growth rate (SGR) than BFAR but no differences in final weight, yield, and feed conversion ratio (FCR).

Although CAL showed slightly better survival than BFAR, the difference was not significant.

For the second run, survival, SGR, and FCR were significantly better in CAL than BFAR. Like the first run, there were no significant differences in the final weight of the two strains.



Culture of prawn in Laguna de Bay for evaluation of growth performance





### Reproductive Efficiency of Two *M. rosenbergii* Stocks at Different Protein Levels

Spawning sets (1 male:5 females) of four-month old *Macrobrachium rosenbergii* from a hatchery stock (BFAR strain *M. rosenbergii rosenbergii*) and a wild stock (Calumpit strain, possibly *M. rosenbergii rosenbergii* x *Mr dacqueti* F<sub>1</sub>s) were placed in replicate 2 x 2 x 1 outdoor concrete tanks in April 2005. Stocks were fed using the following treatments: Treatment A: low protein (commercial fish feed pellets) at 2% of the prawn biomass; Treatment B: high protein (prawn feed pellets) at 2% of the prawn biomass and Treatment C: low protein (commercial fish feed pellets), given *ad libitum*.



AQD's Dr. Ma. Lourdes Aralar reporting on the results of freshwater prawn studies conducted in Lake Laguna de Bay

Six months after stocking, preliminary observation showed that the BFAR stock fed low protein diet (fish feed pellets) *ad libitum* spawned more frequently than prawns given fixed amounts of fish feed and prawn feed. Calumpit stocks spawned less frequently: 9 (treatment C), 8.7 (treatment A) and 6 (treatment B) spawning episodes. The average number of hatchlings produced per gram body weight of the female prawn broodstock was highest in the BFAR stocks at 669.7 (for treatment C), 665.28 (for treatment B) and 567.2 (for treatment A). The same ranking was observed in the Calumpit stock at 598.4, 532.7 and 438.7, respectively.

In a similar experiment set up in lake-based net cages using five-month old prawns to determine if the reproductive efficiency of the two strains is influenced by the type of spawning system, the survival of post-larvae produced from the spawns was low in both the BFAR and Calumpit stocks. The highest postlarval survival rate was achieved in one batch of spawn produced in an earlier trial run conducted in November 2004 (these F<sub>1</sub>s were reared further to become parents of the breeders used in the on-going experiment).

### *Macrobrachium rosenbergii* and Other Indigenous *Macrobrachium* species in Mindanao and Visayan Islands

Specimens of adult and juvenile *Macrobrachium* were collected from six known prawn grounds in different parts of Mindanao (Philippines). The description of the specimens formed as basis for its taxonomic identification, and the growth and survival of *Macrobrachium rosenbergii* was determined using various culture systems and compared the performance of wild and hatchery-bred prawn fry.

The prawns in hapa nets were fed commercial shrimp pellets at 10% body weight. The results of the study showed that *M. rosenbergii dacqueti* is a fast growing subspecies compared to *M. rosenbergii rosenbergii*. The observation was evident even before the experiment was started.

The discrepancies in size between the two species were even prominent in the final weights. On survival however, *M. rosenbergii dacqueti* had lower rate upon termination of the study. This is attributed to the mass mortality observed when plankton bloom collapsed.

Apart from continuing these aforementioned research activities, more studies related to the domestication (refinement of nursery and grow-out technologies) and performance assessment of wild freshwater prawn stocks/species will be done in the next phase of the project before any efforts to genetically improve growth and survival in existing hatchery stocks can be undertaken.



Dr. Henry Dejarme of MSU reporting on the results of the prawn study conducted in Mindanao